INDUCED SUPERSATURATION BY HYDROXYPROPYLMETHYL CELLULOSE UNDER AQUEOUS ACIDIC CONDITIONS OF A SERIES OF CARBOXYLIC ACID DRUGS

By

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Salina Liao

Submitted to the graduate degree program in Pharmaceutical Chemistry and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master's of Science.

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that this is the approved Version of the following thesis:

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Chairperson

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Valentino J.Stella

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Date approved

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1. TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

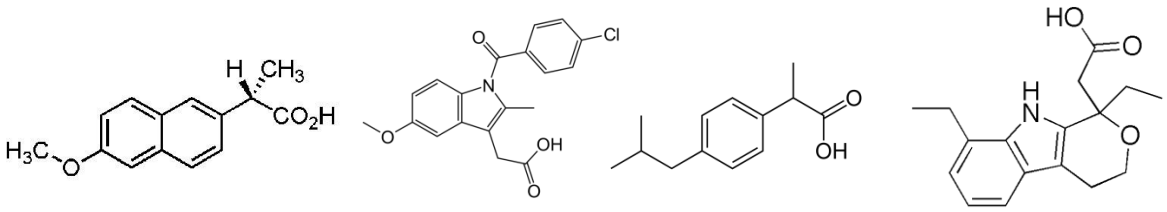
2. ABSTRACT

Supersaturation of four structurally related crystalline carboxylic acid drugs (naproxen, indomethacin, ibuprofen, and etodolac) was studied in aqueous acidic conditions at 37ºC in the absence and presence of pre-dissolved HPMC. A solvent-shift method was used in which drug-containing DMSO solution concentrates were prepared with each molecule and carefully added to aqueous acidic solutions while stirring. Tests were performed to measure drug dissolved concentration as a function of time and to characterize any resulting precipitate. Supersaturation was observed for all four model carboxylic acid drugs in the presence of HPMC-containing aqueous acidic solutions. When precipitation occurred the isolated solid drug was characterized and shown to increase in amorphous character or produce completely amorphous solid. The solution and solid-state data suggests that the mechanisms by which HPMC induces supersaturation include both increased equilibrium solubility and crystallization inhibition in the presence of HPMC in naproxen, indomethacin, and ibuprofen. In etodolac, there was no observed significant increase of equilibrium solubility, and only a loss of crysallinity upon precipitation in kinetic solubility studies. Additional studies with etodolac showed that the grade and viscosity of HPMC did not significantly affect the degree of supersaturation maintained. However, an increased initial degree of supersaturation of etodolac results in a decreased onset time of precipitation. Within the range studied the optimal degree of supersaturation of etodolac was 5 in which no precipitation occurred, at a degree of supersaturation of 7 precipitation occurred after 3 hours, whereas at a degree of supersaturation of 10 precipitation occurred immediately. The studies suggest that the solubilizing and supersaturating-promoting effects of HPMC may be useful in designing preclinical, clinical and commercial formulations and drug products to potentially improve the oral bioavailability of poorly water-soluble compounds.

3. PURPOSE

The purpose of the current work is to explore the variables by which four crystalline carboxylic acid drug molecules show supersaturation behavior in the presence of hydroxypropyl methylcellulose (HPMC) under aqueous acidic pH conditions. Four model compounds were evaluated: naproxen, indomethacin, ibuprofen, and etodolac. The molecular structures are shown in Figure 1. Experiments were performed by carefully adding a concentrated drug in DMSO solution into a stirred aqueous acidic solution with and without dissolved HPMC, and then observing for precipitation, measuring dissolved drug concentration, and characterizing the isolated drug precipitate. All compounds were evaluated at an initial degree of supersaturation of five relative to their equilibrium solubility, and were found to show supersaturation behavior in the presence of HPMC. In addition, the precipitates in the presence of HPMC showed a significant loss in crystallinity relative to the starting crystalline model drugs. The mechanisms by which HPMC promotes supersaturation were found to be due to be mainly crystallization or nucleation inhibition, and in part byincreased equilibrium solubility in the presence of HPMC.

Of the compounds studied, etodolac was selected for further study. Specifically, the effects of various viscosities and grades of HPMC, and various initial degrees of initial supersaturation were evaluated.



a) Naproxen b) Indomethacin c) Ibuprofen d) Etodolac

Figure 1. Chemical structures of (a) naproxen, (b) indomethacin, (c) ibuprofen, (d) etodolac

4. INTRODUCTION

4.1. Brief Literature Review of Supersaturation

The recent large number of poorly water-soluble compounds in the pharmaceutical industrial clinical pipelines has led to an increasing interest in maintenance of supersaturation as a means of improving oral bioavailability. Supersaturation may be achieved and sustained with the "spring and parachute" approach.[1] The "spring" dissolves the drug *in vivo* upon oral administration, while the "parachute" sustains the compound in solution by inhibiting precipitation or nucleation in the gastrointestinal lumen. Precipitation must be inhibited for sufficient time on the order of hours in order for the drug to be absorbed after oral administration.[1] The mechanism of supersaturation has been studied extensively. Supersaturation by polymeric ingredients occurs by inhibiting precipitation by two mechanisms: 1) suppressing the drug nucleation process by diffusion-controlled nucleation inhibition and/or 2) adsorption of polymer onto the crystalline surface, thereby preventing solute molecules from incorporating into the growing crystal lattice. The specific supersaturation enhancing properties of polymers, such as HPMC, and the mechanisms by which they occur continue to be explored. HPMC appears to have the ability to enhance solubility and bioavailability. Ouellet et al.[2] reported up to two times increase in oral bioavailability of solid dabrafenib when administered with HPMC capsules compared to gelatin capsules. Upon *in-vivo* dissolution of the HPMC capsule shell in the stomach a solution microenvironment is created that is thought to aid in the dissolution of the solid drug and then upon further dilution in the intestinal tract the presence of HPMC keeps the drug in solution by promoting supersaturation, which ultimately allows for higher absorption compared to gelatin capsules.

Yang et al.[3] observed prolonged supersaturation in a hydrocortisone/HPMC system, in which the HPMC induced the formation of a metastable crystal polymorph. Raina et al.[4] studied the crystallization tendencies of six analogues in the dihydropyridine class in the presence of various polymers. It was found that polymers with intermediate hydrophilicity/hydrophobicity substantially delayed crystallization, while strongly hydrophobic or hydrophilic polymers were generally ineffective. Chauhan et al.[5] observed a correlation between polymers' ability to inhibit

precipitation in solution and amorphous stabilization in solid state for indomethacin. It was found that the rank order of polymer precipitation inhibitory effect for indomethacin was polyvinylpyrrolidone K90 > Eudragit (methacrylic acid) E100 > HPMC. This rank order was the same in the amorphous stabilization. Infrared (IR) and Raman spectroscopy, and solid state NMR showed that the rank order correlated with the strength of the drug polymer interaction.

Petrusevška et al.[6] used the solvent shift method to screen various excipients for precipitation inhibition of sirolimus in neutral pH buffered water. HPMC 603 and Poloxamer 407 were identified as the most effective precipitation inhibitors at 0.1% (w/v) excipient concentration in aqueous pH 6.8 buffer. It was also observed that Poloxamer 407 increased the thermodynamic solubility of sirolimus, whereas the addition of HPMC 603 did not affect the thermodynamic solubility. This difference is explained by the different mechanism by which the excipients inhibit precipitation. The tested concentration of Poloxamer 407 was above the critical micelle concentration, and the increased thermodynamic solubility was likely a result of sirolimus entrapment within micelles. In the presence of HPMC 603, the precipitation inhibition was a result of the polymer adsorbing to the surface of crystal's surface, preventing crystal growth.

The mechanism by which supersaturation is maintained continues to be explored. Patel et al. describe the two-step diffusion-reaction model for crystal growth. The steps comprise of 1) bulk diffusion from the solution to the crystal surface and 2) surface integration such that the solute is incorporated into the growing crystal lattice. At a high degree of supersaturation (degree of supersaturation = 6), it was shown that the crystal growth kinetics of indomethacin was bulk-diffusion controlled. This was shown by comparing the mass transfer coefficients from indomethacin dissolution and crystal growth.[7] It was also found that the growth crystal kinetics of indomethacin is affected by the degree of supersaturation. At high degrees of supersaturation (degree of supersaturation between 6 and 9) , the crystal growth rate coefficient was similar to that of bulk diffusion-controlled crystal growth. At lower degrees of supersaturation (degree of supersaturation = 2), the crystal growth rate coefficient was nearly 10 times less than that of bulk diffusion-controlled crystal growth. The decreased crystal growth rate coefficient could be explained by a change in the rate-limiting step from bulk diffusion to surface integration. Additionally, Patel et al. observed an increase in apparent solubility after crystal growth in samples at degrees of saturation between 2 and 6, which could indicate a higher energy surface

layer had been deposited on the surface of the seed crystals.[8] See additional references [9-21] which the reader might find useful.

4.2. Choice of Model Compounds

Four structurally related carboxylic acids of the nonsteroidal anti-inflammatory class were chosen as model compounds: naproxen (NAP), indomethacin (IND), ibuprofen (IBU), and etodolac (ETO).

4.3. Specific Goals

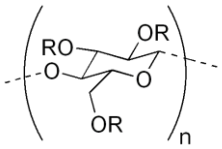
With the increased number of poorly soluble compounds, supersaturation has played an important role in enhancing oral bioavailability of poorly water soluble, dissolution limited drugs. A greater understanding of the mechanisms and role of polymers that promote supersaturation could impact formulation selection in preclinical (PK, toxicology or efficacy) studies, clinical studies as well as commercial formulations/products. The specific goals of this study are the following:

- Explore the mechanism and variables by which model compounds show supersaturation behavior in the presence of HPMC in aqueous solutions
- Explore effects of various grades and viscosities of HPMC on etodoloac solubility and supersaturation
- Explore effects of increased initial degrees of supersaturation on etodolac precipitation behavior.

5. MATERIALS

5.1. Model Compounds

Naproxen was purchased from Sigma-Aldrich Company (St. Louis, Missouri, USA). Indomethacin and ibuprofen were purchased from Enzo Life Sciences (Farmingdale, New York, USA). Etodolac was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Dimethylsulfoxide (DMSO) was purchased from Spectrum (New Brunswick, New Jersey). Hydroxypropyl methylcellulose (grades E3 LV, E5 LV, K3 LV, K100 LV, A 4M, and K 4M) were supplied from Dow Chemical (Pittsburg, California, USA). Hydrochloric acid, 1.0 normal (N) was purchased from J.T. Baker (Center Valley, Pennsylvania, USA). Methanol, acetonitrile, 0.1% TFA solution in water used in this work were HPLC grade and were obtained from Sigma-Aldrich Company (St. Louis, Missouri, USA). The chemical structures of the model compounds and HPMC are shown in Figure 1 and Figure 2, respectively. The properties of the evaluated grades of HPMC are shown in Table 1. The grades of HPMC were chosen based on the filtering capabilities of 5% HPMC solutions.



R = H or CH_3 or $CH_2CH(OH)CH_3$

Figure 2. Structure of hydroxypropyl methylcellulose (HPMC)

Table 1. Properties of evaluated HPMC grades

HPMC Grade	Viscosity (mPa s) in 2% solution in H_2O at 20 ºC	Methoxy Substitution, %	Hydroxypropyl Substitution, %
E3 LV	3	29	8.5
E5 LV	5	29	8.5
K3 LV	3	22	8.1

6. EXPERIMENTAL METHODS

6.1. Preparation of 0.01 N Hydrochloric Acid Solution

Water with hydrochloric (HCl) acid at pH 2 was prepared by performing a 100-fold dilution of 1 M HCl solution into deionized water.

6.2. Preparation of Hydroxypropyl Methylcellulose (HPMC) in 0.01 N HCl Acid Solution

Solutions with various HPMC concentrations in 0.01 N HCl aqueous solution were prepared on weight by weight basis. HPMC was weighed out in appropriate amounts and added to 0.01 N HCl aqueous solution to achieve the desired concentration. The mixture was stirred until the HPMC dissolved. HPMC solutions were filtered through a 0.45 μm polyethersulfone filter, to remove particulates.

6.3. Crystalline Equilibrium Solubility

Equilibrium solubility was evaluated by adding crystalline active pharmaceutical ingredient (API) to each media with 1% DMSO and sonicating for 10 minutes. The reason for the 1% DMSO was to simulate kinetic solubility experimental conditions in which as DMSO concentrate is diluted 100-fold. The suspensions were shaken at 750 rpm in an Eppendorf Thermomixer R (Hamburg, Germany) at 37 °C for up to 1 day and then centrifuged for 10 minutes at 13,200 rpm. The supernatant was removed, diluted 10-times with an HPLC injection solvent composed of either acetonitrile/water (50:50) or ethanol/water (50:50). Standards for each compound were prepared by diluting a 1 mg/mL stock solution in DMSO into an HPLC injection solvent to achieve concentrations of 1 μg/mL, 10 μg/mL, and 100 μg/mL of API. The concentrations of samples and standards were analyzed using the method described in the section “Concentration Determination by High Performance Liquid Chromatography (HPLC)”.

6.4. Preparation of Supersaturated Solutions/ Kinetic Solubility Studies

Kinetic solubility of each API in various media was determined using the solvent-shift method.[1] The crystalline API was dissolved in DMSO at various concentrations, and these stock solutions were carefully diluted 100-fold into 30 mL of aqueous solution at 37 °C with constant stirring. In the evaluated supersaturation method, samples were taken at various time points with a syringe, filtered through a GHP (Hydrophilic polypropylene) membrane filter for naproxen and etodolac, cellulose membrane filter for indomethacin, nylon filter for ibuprofen, and diluted with acetonitrile/water (50:50) or ethanol/water (50:50). The concentrations of samples and standards were analyzed using the method described in the section "Concentration Determination by High Performance Liquid Chromatography (HPLC)".

6.4.1. Calculation of Degree of Supersaturation

The degree of supersaturation (*DS*) of compound is defined as the measured drug concentration at a time point (C_t) relative to the equilibrium solubility of crystalline compound (C_{eq}) in the same media. For supersaturation experiments in the presence of HPMC, C_{eq} is the equilibrium solubility of the crystalline compound in 0.01 N with 5% HPMC. For supersaturation experiments without HPMC, C_{eq} is the equilibrium solubility of the crystalline compound in 0.01 N.

$$DS = \frac{C_t}{C_{eq}} \qquad \text{Equation 1}$$

6.5. Concentration Determination by High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) analyses were performed using a Waters Alliance system comprising of a 2695 Separation Module and model 2996 photodiode array detector (Waters Alliance Instruments, Milford, Massachusetts). The column used was Gemini 5u C18 110A 150 x 4.60 mm, 5 micron supplied by Phenomenex (Torrance, California). The injection volume was 10 μL. Detection was by ultraviolet (UV) absorbance at 232 nm for naproxen, 256 nm for indomethacin, and 226 nm for etodolac and ibuprofen. Empower (Waters Alliance Instruments, Milford, Massachusetts) analysis software was used to integrate peak area.

The aqueous mobile phase was 0.1% TFA in water, and the organic mobile phase was methanol and acetonitrile in a 70:30 (v/v) ratio. The flow rate was 1 mL/min. Elution was performed using the solvent gradient from 20% to 100% organic mobile phase in aqueous mobile phase over 10 minutes.

6.6. Solid State Characterization by X-Ray Powder Diffraction (XRPD)

The solids from the equilibrium solubility studies and supersaturation/kinetic solubility studies were harvested by filtration. Solids from equilibrium solubility studies were collected after shaking for 1 day at 37 ºC. In order to collect remaining solids from kinetic solubility studies, separate kinetic solubility studies were set up at an initial degree of supersaturation of 25 or 50 to force and ensure sufficient precipitation. Remaining suspensions from studies were centrifuged at 13,200 rpm for 10 minutes through a 0.45 μm nylon filter. In the kinetic solubility studies any solid material that precipitated was collected at the 30 minute time point, 3 hour time point, and 24 hour time point (when applicable). Solid samples were analyzed by X-ray powder diffracometer for crystallinity and polymorphic form. In a typical XRPD experiment, powder was deposited in the hollow of an aluminum holder equipped with a zero background plate with the aid of glass slide. Diffraction data (Cu Kα, $\lambda = 1.5418$ Å) were collected on a PANanalytical XPERT-PRO (PANalytical B.V., Almelo, Netherlands) diffractometer. The generator was operated at 45 kV and 40 mA. Slits used: Soller 0.02 rad., antiscatter 1.0°, and divergence. Scans were performed from 2 to 40° 2θ with 0.0084° step size with varying scan rates. Data analysis was performed by X'Pert data viewer Version 1.2d (PANalytical B.V., Almelo, Netherlands).

7. RESULTS

7.1. Equilibrium Solubility of Crystalline API in Absence and Presence of 5% HPMC E3 LV in 0.01 N HCl in Water at 37 °C with 1% DMSO

The equilibrium crystalline solubility of NAP, IND, IBU, and ETO were determined in the absence and presence of 5% HPMC E3 LV pre-dissolved in 0.01 N HCl with 1% DMSO. The solubility results are shown in Table 2. The presence of HPMC enhances the equilibrium solubility of NAP by about 2-fold (26 to 60 μg/mL), of IBU by about 1.6-fold (54 to 86 μg/mL), and 4-fold for IND (2 to 8 μg/mL). There was smaller increase in equilibrium solubility (109 to 123 μg/mL) for ETO in the presence of HPMC compared to the other three acids.

Table 2. Equilibrium solubility values of four crystalline APIs in the absence and presence of 5% HPMC E3 LV in 0.01 N HCl with 1% DMSO in water at 37 ºC

	Equilibrium Solubility of Crystalline API (μg/mL)	
API	0.01 N HCl	5% HPMC E3 LV in 0.01 N HCl
Naproxen	26	60
Indomethacin	2	8
Ibuprofen	54	86
Etodolac	109	123

7.2. Solid-State Characterization by XRPD on Remaining Solid in Equilibrium Solubility Studies

The remaining solid from equilibrium solubility studies in the absence and presence of 5% HPMC E3 LV in 0.01 N HCl were collected and analyzed for polymorphic form change by XRPD for all model compounds. In the case of etodolac, additional HPMC grades were evaluated (still at 5%): HPMC E5 LV and HPMC K3 LV. Figure 3, Figure 4, Figure 5, and Figure 6 show the XRPD scans for NAP, IND, IBU, and ETO, respectively, and each figure has the XRPD of the initial crystralline API and the remaining solid after the equilibrium solubility

measurements. For all compounds, the remaining solids in all equilibrium solubility studies were crystalline and identical to the initial crystalline form.

7.2.1. **Naproxen**

Figure 3. PXRD scans of naproxen (a) initial crystalline naproxen, harvested from equilibrium solubility studies at 37 °C in water with (b) 0.01 N HCl, and (c) 0.01 N HCl with 5% HPMC E3 LV

a

7.2.2. **Indomethacin**

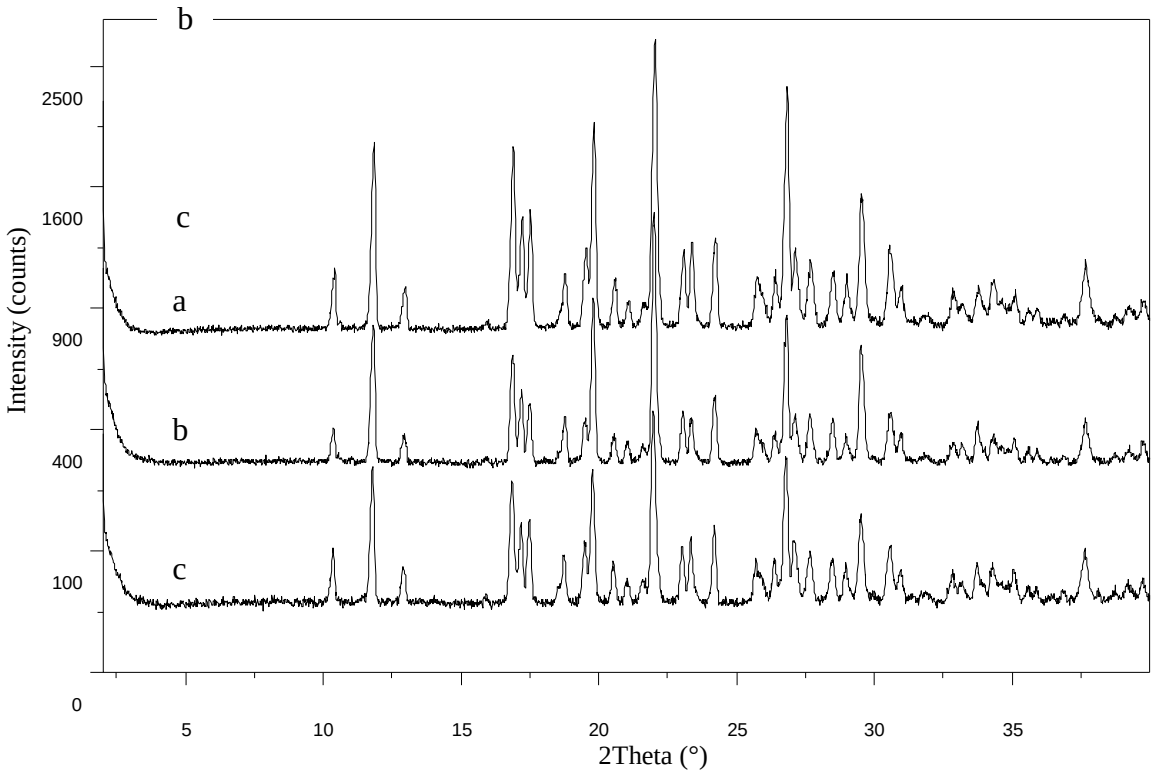


Figure 4. PXRD scans of indomethacin (a) initial crystalline indomethacin, harvested from equilibrium solubility studies at 37 °C in water with (b) 0.01 N HCl, and (c) 0.01 N HCl with 5% HPMC E3 LV

7.2.3. **Ibuprofen**

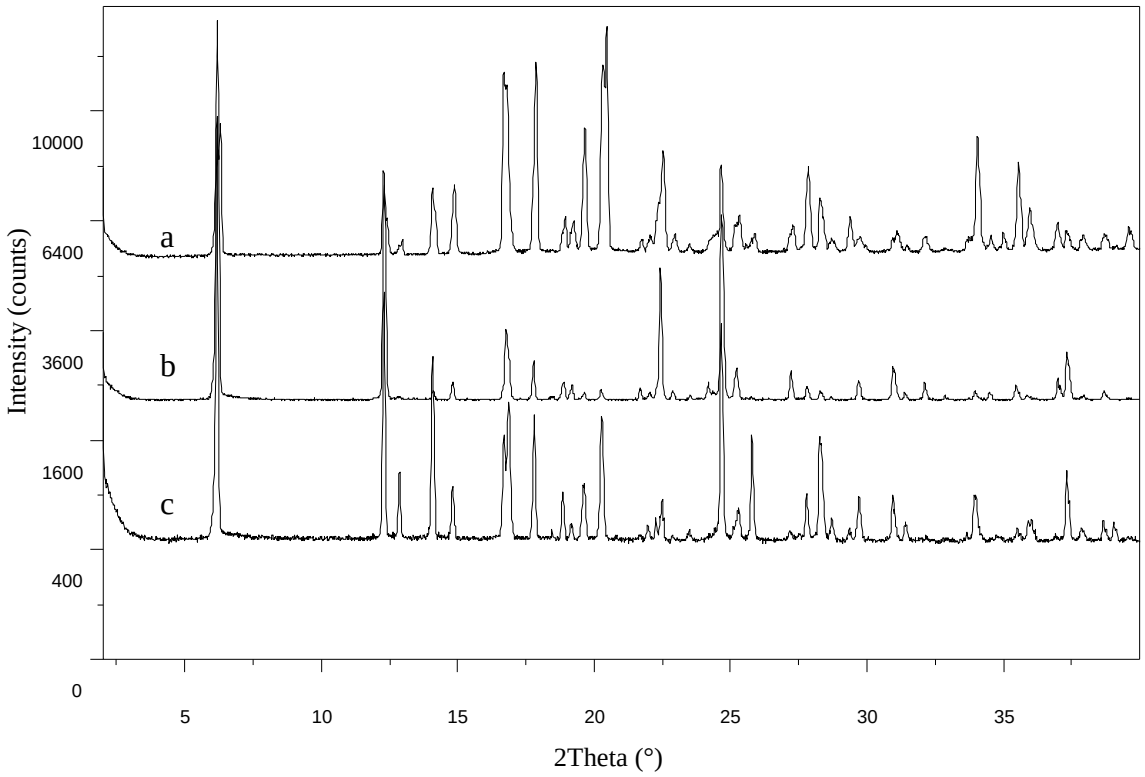


Figure 5. PXRD scans of ibuprofen (a) initial crystalline ibuprofen, harvested from equilibrium solubility studies at 37 °C in water with (b) 0.01 N HCl, and (c) 0.01 N HCl with 5% HPMC E3 LV

7.2.4. **Etodolac**

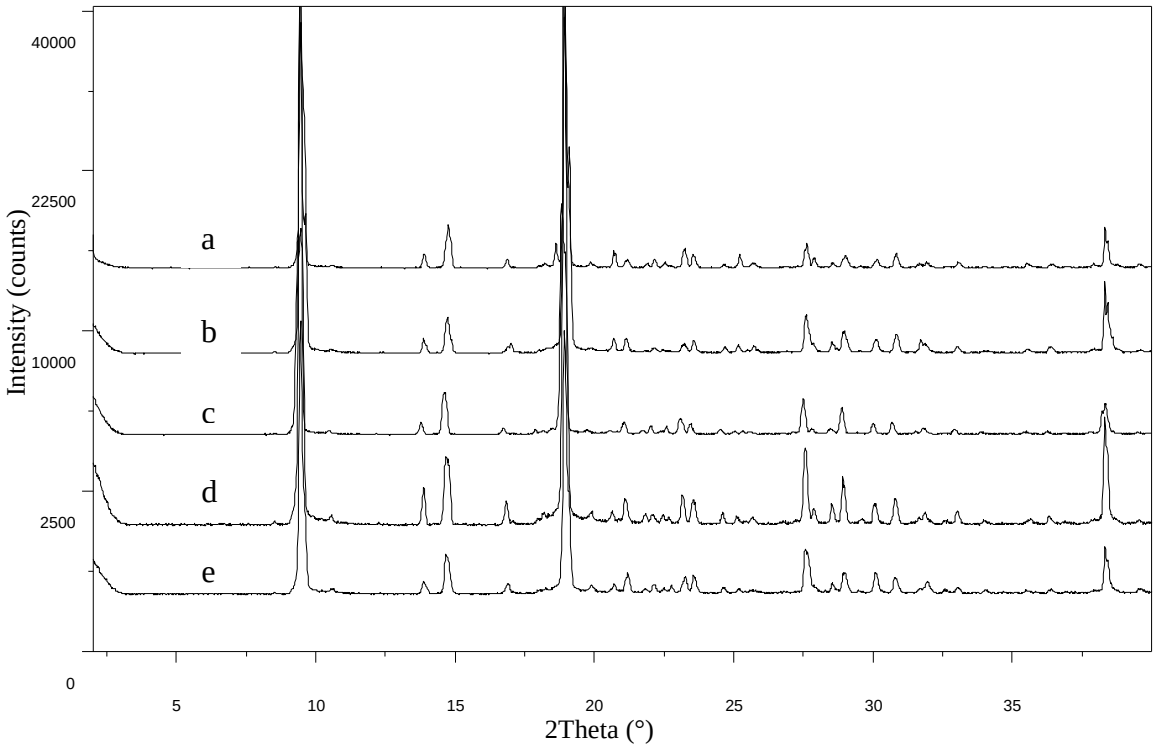


Figure 6. PXRD scans of etodolac (a) neat crystalline etodolac, harvested from equilibrium solubility studies at 37 °C in water with 0.01 N HCl (b) without HPMC, (c) with 5% HPMC E3 LV, (d) with 5% HPMC E5 LV, (e) with 5% HPMC K3 LV

7.3. Effect of HPMC on Supersaturation Maintenance

7.3.1. Naproxen

7.3.1.1. Supersaturation Maintenance

Supersaturation maintenance was evaluated for naproxen (NAP) in the presence and absence of 5% HPMC E3 LV in water with 0.01 N HCl at 37 ºC for up to 3 hours. Figure 7 shows the concentration-time profiles and supersaturation-time profiles for NAP. The initial degree of supersaturation was 5. In the absence and presence of HPMC E3 LV there was observed immediate precipitation of NAP. In the absence of HPMC the dissolved concentration of NAP immediatey decreased but was still above the equilibrium solubility value and a degree of supersaturation of approximately 1.5 was maintained throughout the course of the experiment. In the presence of HPMC E3 LV the dissolved concentration of NAP immediatey decreased but

was still above the equilibrium solubility value and a degree of supersaturation of approximately 2.3 was observed at 30 minutes, and 1.9 after 3 hours..

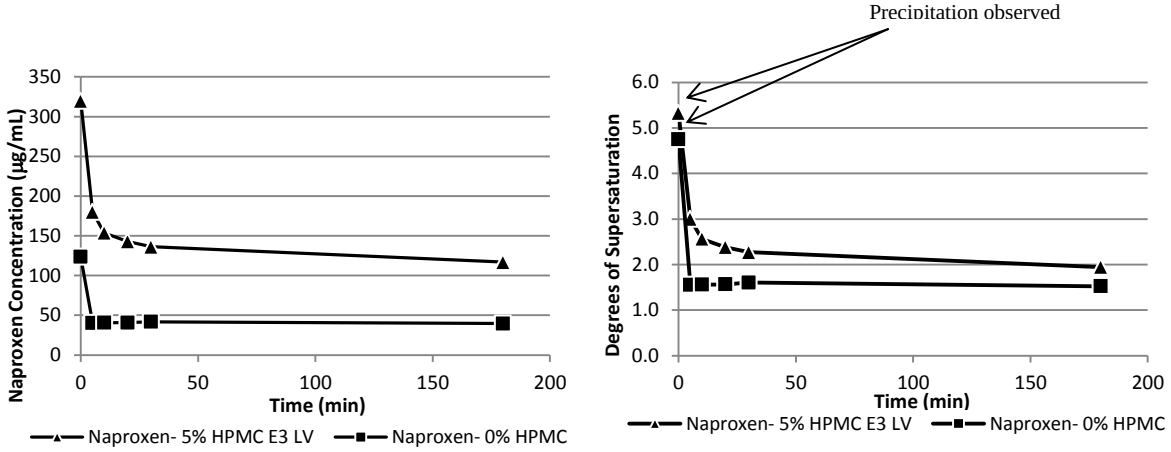


Figure 7. Concentration-time profiles (left) and supersaturation-time profiles (right) of dissolved naproxen in water in the absence (■) and presence (▲) of 5% HPMC E3 LV in 0.01 N HCl and 1% DMSO at 37 ºC. Total naproxen concentration was 130 µg/mL in the absence of HPMC E3 LV and 300 µg/mL in the presence of HPMC E3 LV. The initial degree of supersaturation was 5.

7.3.1.2. Solid-state Characterization by XRPD

The precipitated solids from NAP kinetic solubility studies under forced precipitation conditions (refer to Section 6.6) were harvested by filtration and analyzed for crystallinity by XRPD.

Figure 8 compares the XRPD scans of initial crystalline NAP, precipitated solid material from kinetic solubility studies in in the absence and presence of 5% HPMC E3 LV in 0.01 N HCl at 30 minutes and 3 hours of the experiment. The results show the precipitated solid in the absence and presence of HPMC E3 LV is of a different form than the initial crystalline form of NAP. In the absence of HPMC E3 LV, precipitated NAP shows to be a different crystalline form than the intial form. Based on the lack of sharpness in the later peaks, not only might a new polymorph of naproxen be formed but some amorphous characteristics are seen in the solids in the presence of HPMC E3 LV.

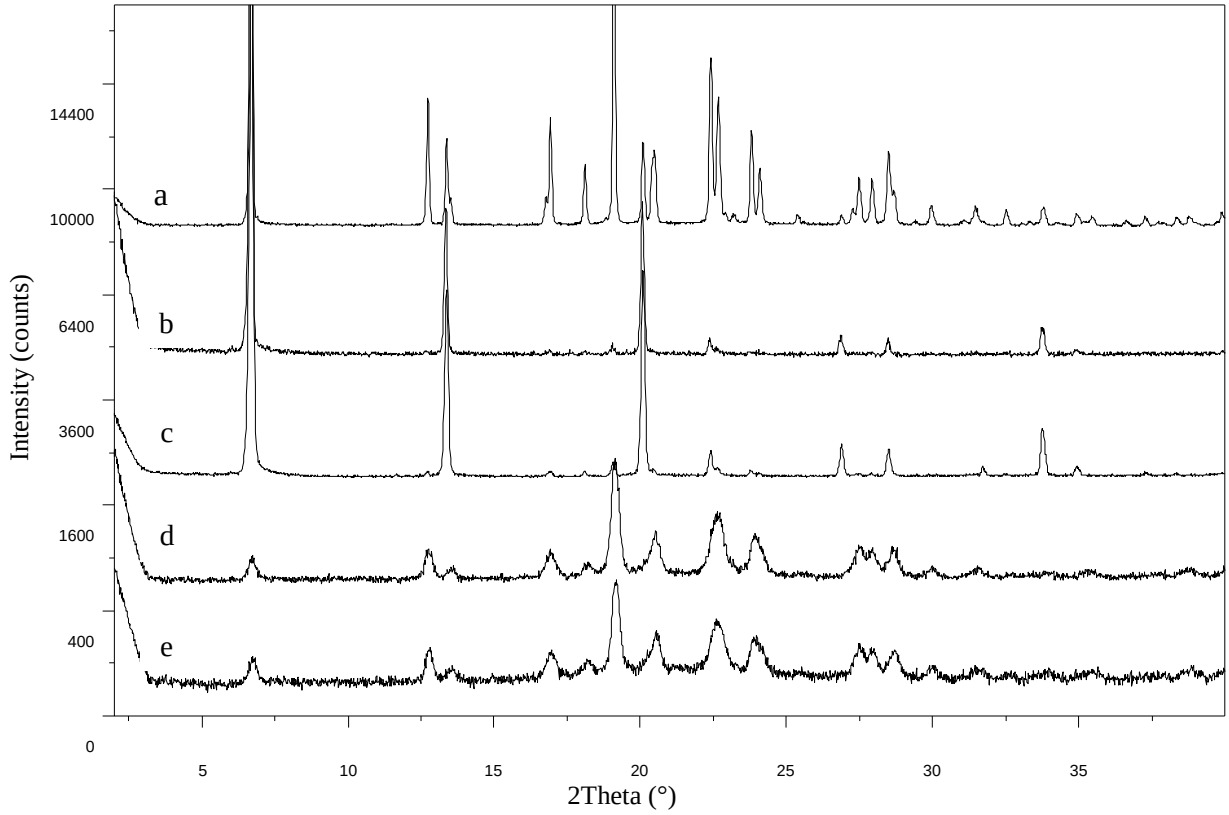


Figure 8. PXRD scans of naproxen (a) neat crystalline naproxen harvested from kinetic solubility studies under forced precipitation conditions at 37 °C in water with (b) 0.01 N HCl at 30 minutes, (c) 0.01 N HCl at 3 hours, (d) 0.01 N HCl with 5% HPMC E3 LV at 30 minutes, (e) 0.01 N HCl with 5% HPMC E3 LV at 3 hours

7.3.2. **Indomethacin**

7.3.2.1. Supersaturation Maintenance

Supersaturation maintenance was evaluated for indomethacin (IND) in the presence and absence of 5% HPMC E3 LV in water with 0.01 N HCl at 37 ºC for up to 3 hours. Figure 9 shows the concentration-time profiles and supersaturation-time profiles for IND. The initial degree of supersaturation was 5. In the absence of HPMC there was no IND precipitation for 30 minutes, but after 3 hours precipitation occurred, and the dissolved IND concentration was above the equilibrium solubility with a degree of supersaturation of approximately 2.5. In the presence of

HPMC E3 LV there was no observed precipitation of IND and the degree of supersaturation of 5 was maintained throughout the course of the experiment.

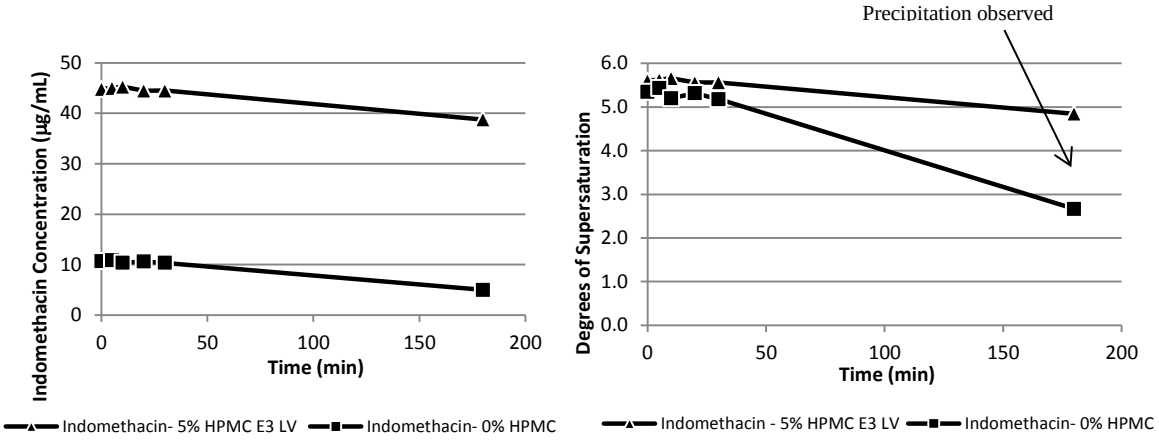


Figure 9. Concentration-time profiles (left) and supersaturation-time profiles (right) of dissolved indomethacin in water in the absence (■) and presence (▲) of 5% HPMC E3 LV in 0.01 N HCl and 1% DMSO at 37 ºC. Total indomethacin concentration was 10 µg/mL in the absence of HPMC E3 LV and 40 µg/mL in the presence of HPMC E3 LV. The initial degree of supersaturation was 5.

7.3.2.2. Solid-state Characterization by XRPD

The precipitated solids from IND kinetic solubility studies under forced precipitation conditions (refer to Section 6.6)were harvested by filtration and analyzed for crystallinity by XRPD. Figure 10 compares the XRPD scans of initial crystalline IND, precipitated solid material from kinetic solubility studies in the absence and presence of 5% HPMC E3 LV in 0.01 N HCl at 30 minutes and 3 hours of the experiment. The results show that the IND precipitated solid forms in the absence and presence of HPMC E3 LV are amorphous.

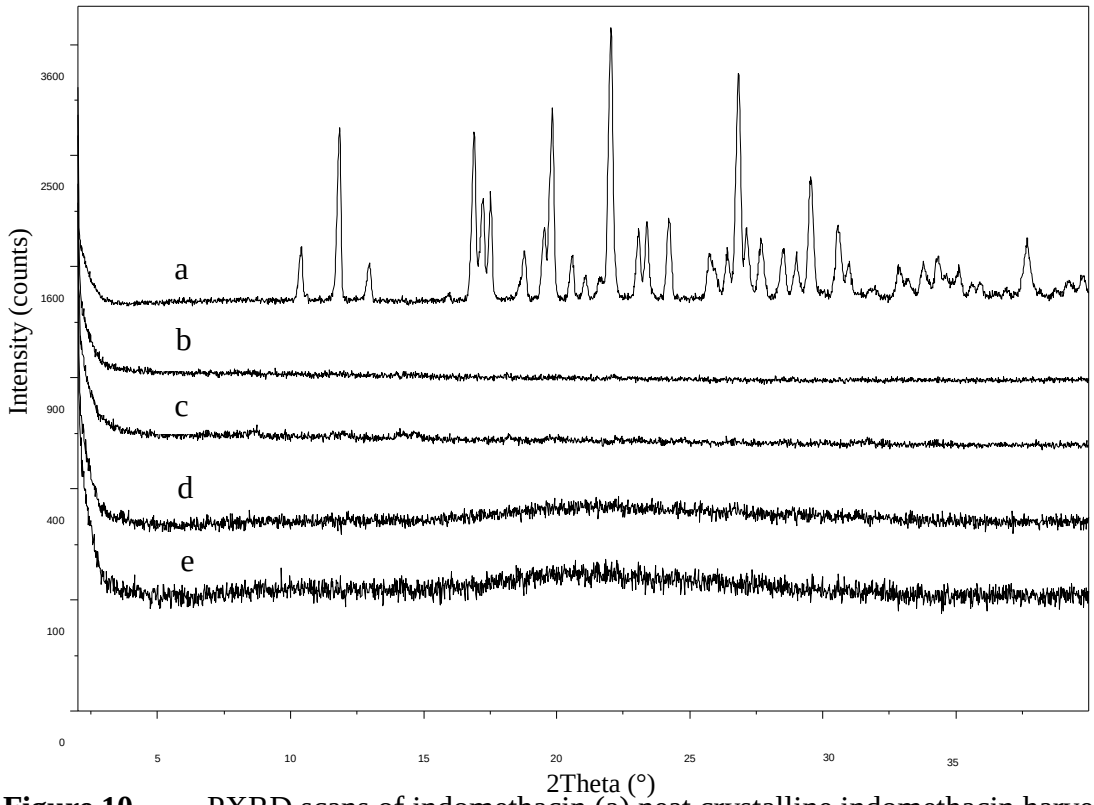


Figure 10. PXRD scans of indomethacin (a) neat crystalline indomethacin harvested from kinetic solubility studies under forced precipitation conditions at 37 °C in water with (b) 0.01 N HCl at 30 minutes, (c) 0.01 N HCl at 3 hours, (d) 0.01 N HCl with 5% HPMC E3 LV at 30 minutes, (e) 0.01 N HCl with 5% HPMC E3 LV at 3 hours

7.3.3. **Ibuprofen**

7.3.3.1. Supersaturation Maintenance

Supersaturation maintenance was evaluated for ibuprofen (IBU) in the presence and absence of 5% HPMC E3 LV in water with 0.01 N HCl at 37 ºC for up to 3 hours. Figure 11 shows the concentration-time profiles and supersaturation-time profiles for IBU. The initial degree of supersaturation was 5. In the absence and presence of HPMC E3 LV there was observed immediate precipitation of IBU. In the absence of HPMC the dissolved concentration of IBU immediatey decreased to the equilibrium solubility value within 10 minutes. In the presence of HPMC E3 LV the dissolved concentration of IBU immediately decreased but was still above the

equilibrium solubility value and a degree of supersaturation of approximately 3.0 was maintained throughout the course of the experiment..

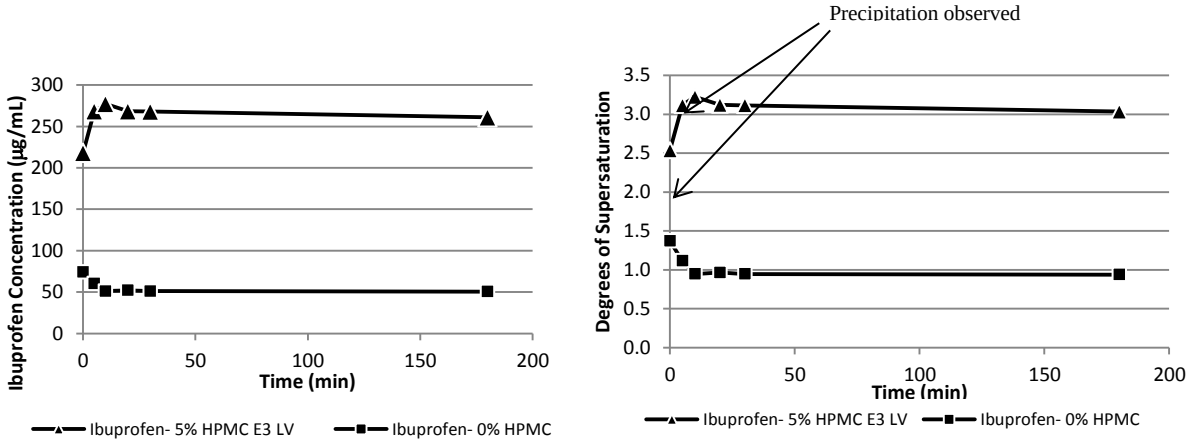


Figure 11. Concentration-time profiles (left) and supersaturation-time profiles (right) of dissolved ibuprofen in water in the absence (■) and presence (▲) of 5% HPMC E3 LV in 0.01 N HCl and 1% DMSO at 37 ºC. Total ibuprofen concentration was 250 µg/mL in the absence of HPMC E3 LV and 450 µg/mL in the presence of HPMC E3 LV. The initial degree of supersaturation was 5.

7.3.3.2. Solid-state Characterization by XRPD

The precipitated solids from IBU kinetic solubility studies under forced precipitation conditions (refer to Section 6.6)were harvested by filtration and analyzed for crystallinity by XRPD. Figure 12 compares the XRPD scans of initial crystalline IBU, precipitate from kinetic solubility studies in in the absence and presence of 5% HPMC E3 LV in 0.01 N HCl at 30 minutes and 3 hours of the experiment. The scans show that in the absence of HPMC E3 LV, the precipitated solids are of the same crystalline form as the initial crystalline IBU, although there is some loss of sharpness in some of the peaks. In the presence of HPMC E3 LV, there is some amorphous character in the precipitated solid but the peaks seen in these materials (presumably a mixture) do correspond to the peaks for the known crystalline form of IBU.

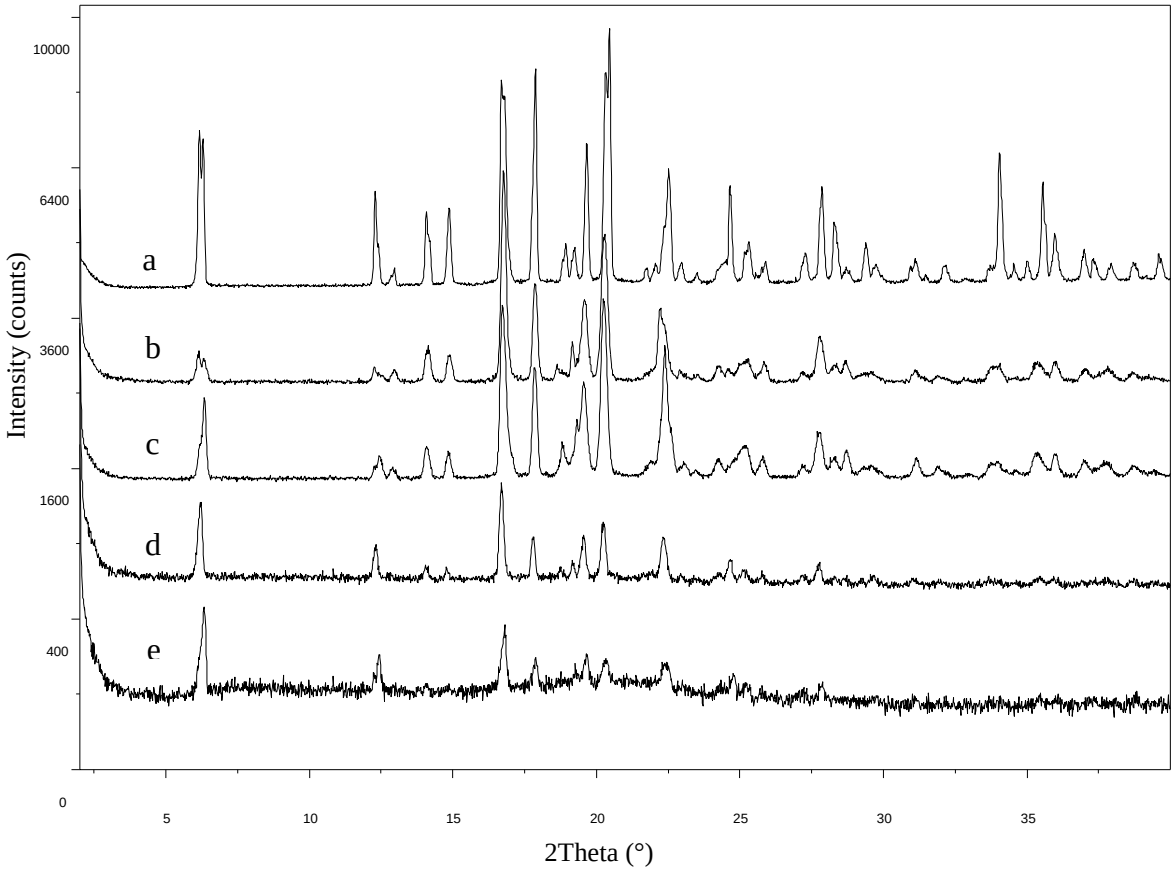


Figure 12. PXRD scans of ibuprofen (a) neat crystalline ibuprofen, harvested from kinetic solubility studies under forced precipitation conditions at 37 °C in water with (b) 0.01 N HCl at 30 minutes, (c) 0.01 N HCl at 3 hours, (d) 0.01 N HCl with 5% HPMC E3 LV at 30 minutes, (e) 0.01 N HCl with 5% HPMC E3 LV at 3 hours

7.3.4. **Etodolac**

7.3.4.1. Supersaturation Maintenance

Supersaturation maintenance was evaluated for etodolac (ETO) in the presence and absence of 5% HPMC E3 LV in water with 0.01 N HCl at 37 ºC for up to 3 hours. Figure 13 shows the concentration-time profiles and supersaturation-time profiles for ETO. The initial degree of supersaturation was 5. In the absence of HPMC there was no precipitation for 5 minutes, but after 10 minutes precipitation occured and the dissolved ETO concentration decreased to near the equilibrium solubility value. In the presence of HPMC E3 LV there was no observed

precipitation of ETO and the degree of supersaturation of 5 was maintained throughout the course of the experiment.

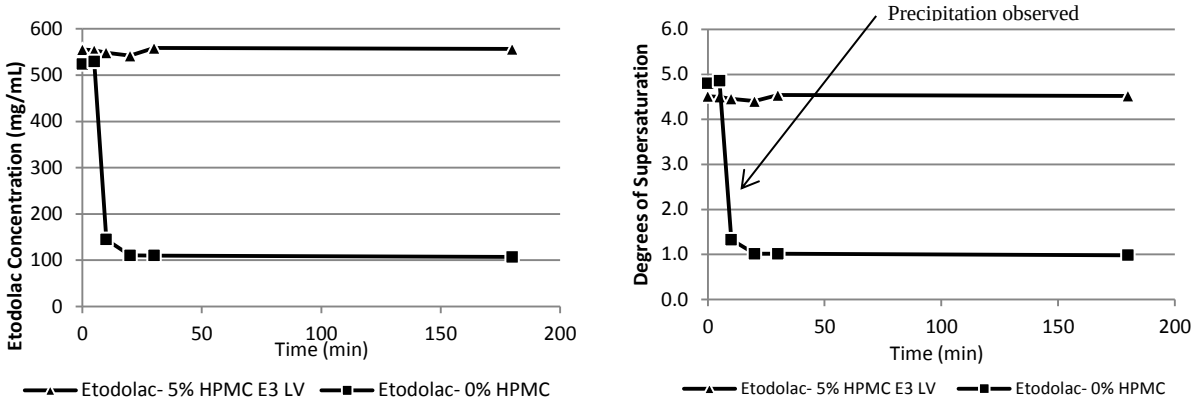


Figure 13. Concentration-time profiles (left) and supersaturation-time profiles (right) of dissolved etodolac in water in the absence (■) and presence (▲) of 5% HPMC E3 LV in 0.01 N HCl and 1% DMSO at 37 ºC. Total etodolac concentration was 550 μg/mL in the absence of HPMC E3 LV and 625 μg/mL in the presence of HPMC E3 LV. The initial degree of supersaturation was 5.

7.3.4.2. Solid-state Characterization by XRPD

The precipitate from ETO kinetic solubility studies under forced precipitation conditions (refer to Section 6.6)were harvested by filtration and analyzed for crystallinity by XRPD.

Figure 14 compares the XRPD scans of initial crystalline ETO, precipitates from kinetic solubility studies in in the absence and presence of 5% HPMC E3 LV in 0.01 N HCl at 30 minutes and 3 hours of the experiment. The scans show that in the absence of HPMC E3 LV, the precipitated solid material shows some crystalline characteristics but is not identical to the initial crystalline form of ETO. In the presence of HPMC E3 LV, the precipitated solid material is amorphous.

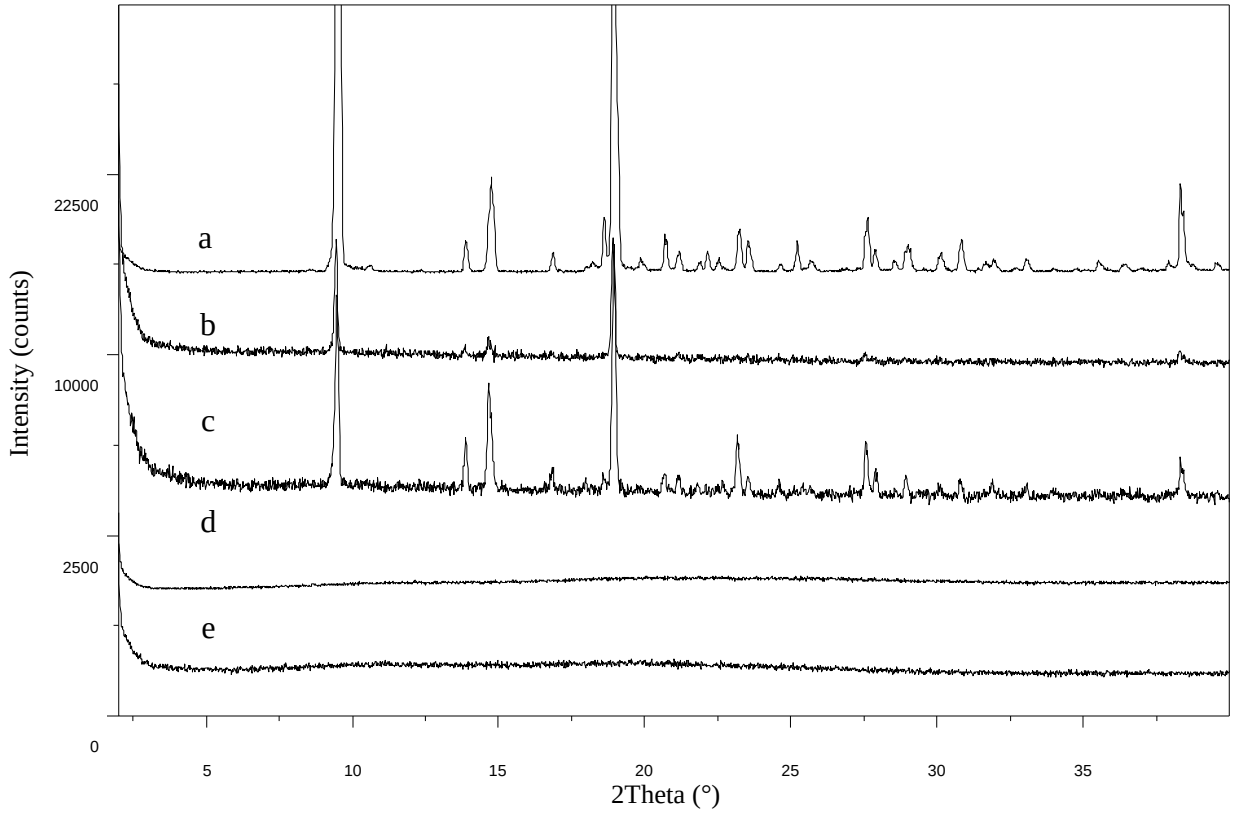


Figure 14. PXRD scans of etodolac (a) neat crystalline etodolac, harvested kinetic solubility studies under forced precipitation conditions at 37 °C in water with (b) 0.01 N HCl at 30 minutes, (c) 0.01 N HCl at 3 hours, (d) 0.01 N HCl with 5% HPMC E3 LV at 30 minutes, (e) 0.01 N HCl with 5% HPMC E3 LV at 3 hours

7.4. Effect of Various Grades of HPMC on Supersaturation Maintenance in Etodolac

7.4.1. Effect of HPMC Grade on Apparent Equilibrium Solubility of Etodolac

The equilibrium solubility of crystalline ETO was determined as a function of various grades of HPMC pre-dissolved at 5% in water with 0.01 N HCl. The equilibrium crystalline solubility values are shown in Table 3. There was some effect on equilibrium solubility of ETO in the presence of the evaluated grades of HPMC. The equilibrium solubility values were 109 μg/mL, 123 μg/mL, 105 μg/mL, and 115 μg/mL in the absence of HPMC, 5% HPMC E3 LV, 5% HPMC E5 LV, and 5% HPMC K3 LV, respectively.

Table 3. Equilibrium solubility values of crystalline etodolac in the absence and presence of various grades of 5% HPMC in 0.01 N HCl in water at 37 ºC with 1% DMSO

Aqueous Media with 1% DMSO	Equilibrium Solubility of Crystalline Etodolac (μg/mL)
0.01 N HCl	109
0.01 N HCl with 5% HPMC E3 LV	123
0.01 N HCl with 5% HPMC E5 LV	105
0.01 N HCl with 5% HPMC K3 LV	115

7.4.2. **Effect of HPMC Grade on Supersaturation of Etodolac**

7.4.2.1. Supersaturation Maintenance in Various Grades of HPMC

Supersaturation maintenance was evaluated for etodolac in the absence and presence of 5% HPMC E3 LV, 5% HPMC E5 LV, and 5% HPMC K3 LV in 0.01 N HCl at 37 ºC for up to 3 hours. Figure 15 shows the concentration-time profiles and supersaturation-time profiles for ETO in the evaluated grades of HPMC. The initial degree of supersaturation was 5. In the absence of HPMC there was no precipitation for 5 minutes, but after 10 minutes precipitation occured and the dissolved ETO concentration near the equilibrium solubility value. In the presence of each grade of HPMC there was no observed precipitation of ETO and the degree of supersaturation of approximately 5 was maintained throughout the course of the experiment.

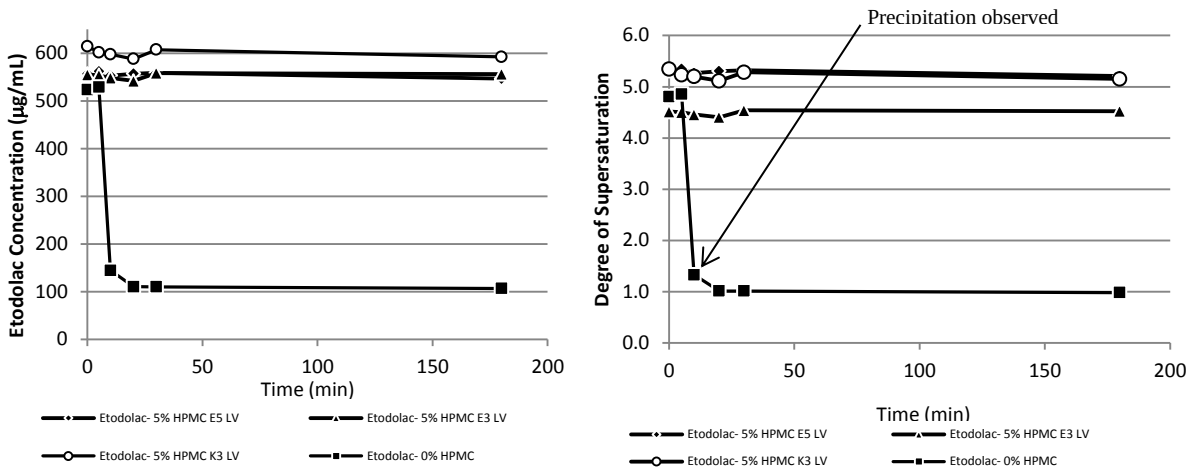


Figure 15. Concentration-time profiles (left) and supersaturation-time profiles (right) of dissolved etodolac in water in the absence (■) and presence of 5% HPMC E3 LV(▲), 5% HPMC E5 LV(♦), and 5% HPMC K3 LV(○) in 0.01 N HCl and 1% DMSO at 37 ºC. Total etodolac concentration was 550 µg/mL in the absence of HPMC and 625 µg/mL in the presence of HPMC. The initial degree of supersaturation was 5.

7.4.2.2. Solid-state Characterization by XRPD

7.4.3. **HPMC E5 LV**

The precipitates from ETO kinetic solubility studies under forced precipitation conditions (refer to Section 6.6)in 5% HPMC E5 LV were harvested by filtration and analyzed for crystallinity by XRPD.

Figure 16 compares the XRPD scans of initial crystalline ETO and the precipitates from the kinetic solubility studies in in the absence and presence of 5% HPMC E5 LV in 0.01 N HCl at 30 minutes and 3 hours of the experiment. The scans show that in the absence of HPMC E5 LV the precipitated solid materials show some crystalline characteristics, but are not polymorphically identical to the initial crystalline form of ETO. In the presence of HPMC E5 LV, the precipitated solid materials are amorphous.

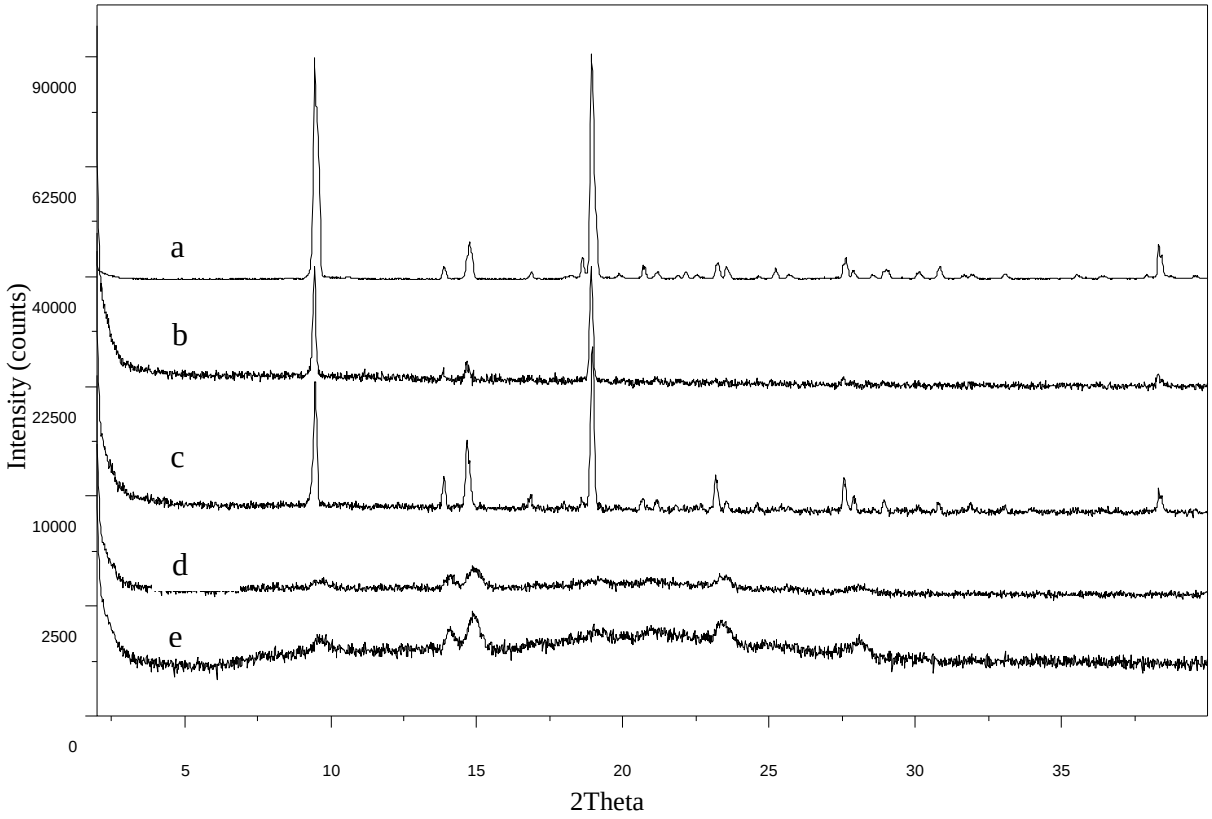


Figure 16. PXRD scans of etodolac (a) neat crystalline etodolac, harvested from kinetic solubility studies under forced precipitation conditions at 37 °C in water with (b) 0.01 N HCl at 30 minutes, (c) 0.01 N HCl at 3 hours, (d) 0.01 N HCl with 5% HPMC E5 LV at 30 minutes, (e) 0.01 N HCl with 5% HPMC E5 LV at 3 hour

7.4.4. **HPMC K3 LV**

The precipitates from ETO kinetic solubility studies under forced precipitation conditions (refer to Section 6.6) in 5% HPMC K3 LV were harvested by filtration and analyzed for crystallinity by XRPD. Figure 17 compares the XRPD scans of initial crystalline ETO and theprecipitates from the kinetic solubility studies in in the absence and presence of 5% HPMC K3 LV in 0.01 N HCl at 30 minutes, 3 hours, and 24 hours of the experiment. The scans show that in the absence of HPMC K3 LV the precipitated solid materials show some crystalline characteristics, but are not identical to the initial crystalline form of ETO. In the presence of HPMC K3 LV the precipitated solid materials shows some amorphous characteristics with some peaks corresponding with the intial starting crystalline material.

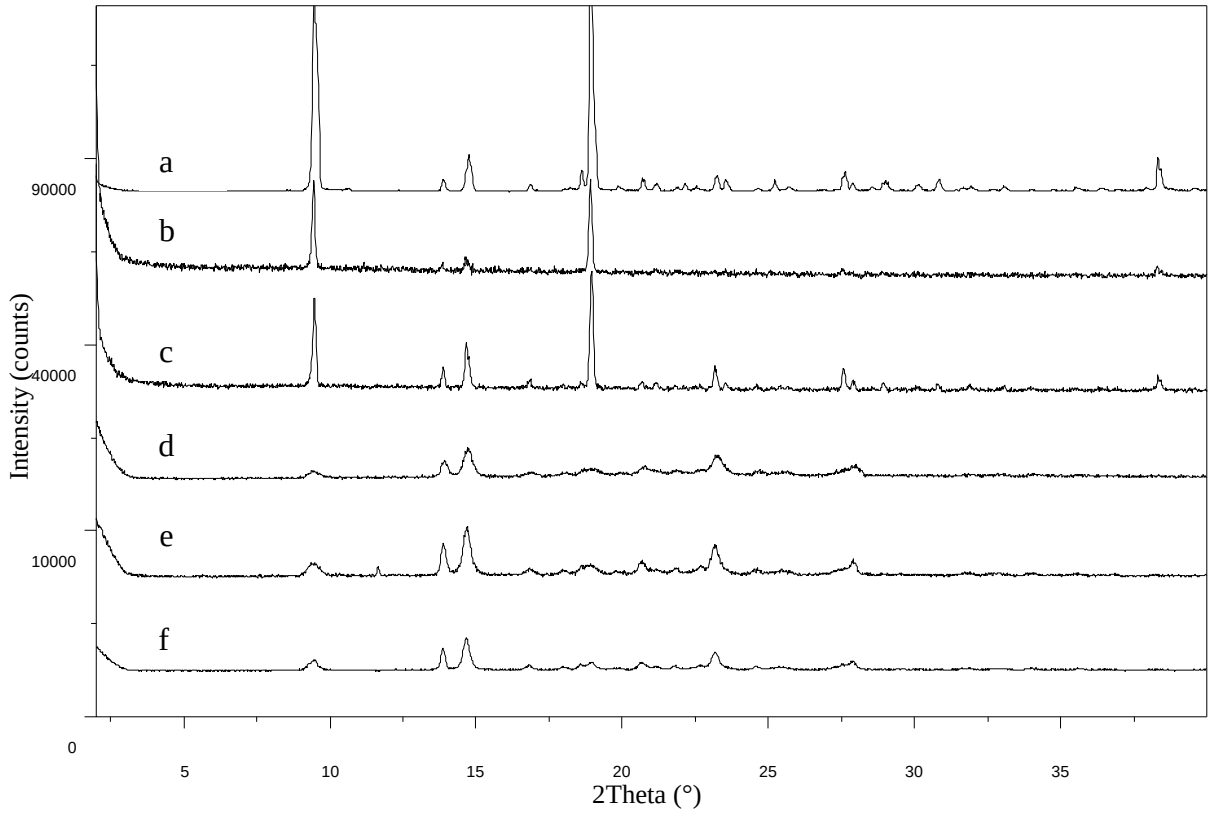


Figure 17. PXRD scans of etodolac (a) neat crystalline etodolac, harvested from kinetic solubility studies under forced precipitation conditions at 37 °C in water with (b) 0.01 N HCl at 30 minutes, (c) 0.01 N HCl at 3 hours, (d) 0.01 N HCl with 5% HPMC K3 LV at 30 minutes, (e) 0.01 N HCl with 5% HPMC K3 LV at 3 hour, (f) 0.01 N HCl with 5% HPMC K3 LV at 24 hour

7.5. Effect of Various Initial Degrees of Supersaturation on Observed Precipitation Time in Supersaturation in Etodolac

Supersaturation maintenance at various initial degrees of supersaturation was evaluated for etodolac in 0.01 N HCl with pre-dissolved 5% HPMC K3 LV at 37 ºC for up to 24 hours. Figure 18 shows the concentration-time profiles and supersaturation-time profiles for ETO at initial degrees of supersaturation of 5, 7, and 10. Precipitation occurred with degrees of supersaturation of 7 and 10, but no precipitation occured with a degree of supersaturation of 5. Table 4 lists the time at which precipitation was observed at various initial degrees of supersaturation. At an initial degree of supersaturation of 5, ETO remains in solution with no precipitation throughout the course of the 24-hour experiment. At an initial degree of supersaturation of 7, ETO remains in solution with no precipitation for 3 hours, but precipitation was observed between 3 and 6

hours with the solution concentration maintained above the equilibrium solubility and degrees of supersaturation of approximately 6 and 3 observed at 6 and 24 hours, respectively. At an initial degree of supersaturation of 10, precipitation of ETO is observed immediately with the solution concentration maintained above the equilibrium solubility and degrees of supersaturation of approximately 3 observed between 3 and 24 hours.

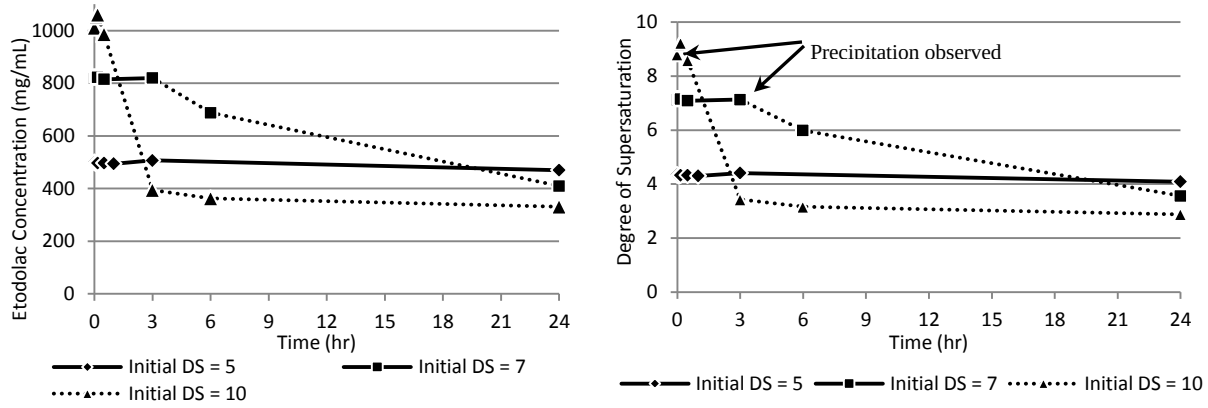


Figure 18. Concentration-time profiles (left) and supersaturation-time profiles (right) of dissolved etodolac in water with 5% HPMC K3 LV and 0.01 N HCl and 1% DMSO at 37 ºC. The initial degrees of supersaturation were 5 (♦), 7 (■), and 10 (▲). Dashed curves indicate time at which precipitation was observed.

Table 4. Time of observed precipitation of etodolac with various initial degrees of supersaturation in water with 5% HPMC K3 LV and 0.01 N HCl and 1% DMSO at 37 ºC

Initial Degree of Supersaturation	Time of Observed Precipitation
5	None up to 24 hours[a]
7	3 hours
10	Immediate

[a] Experiment terminated after 24 hours

8. DISCUSSION

8.1. Increased Equilibrium Solubility of Crystalline API in the Presence of HPMC

The apparent increased equilibrium solubility of crystalline NAP, IND, IBU, and ETO under aqueous acidic conditions with 5% HPMC E3 LV indicates a favorable solution interaction between the unionized drugs studied and HPMC in solution. The favorable interaction could be explained by a decrease in chemical potential of the compounds in solution relative to the chemical potential in the absence of HPMC. Equation 2 shows the relationship between the chemical potential (μ_i) of a chemical species in solution, free energy (G), and the number of dissolved molecules (N_i) in solution at constant temperature (T) and pressure (P). For each of the three compounds, in the equilibrium solubility measurement there was no observed change in polymorphic form in the remaining crystalline solid material compared to the initial crystalline solid, suggesting a constant free energy of the solid during the solubility experiment. The observed increase in solubility of crystalline drugs with pre-dissolved HPMC along with a constant free energy of the crystalline solid means that the chemical potential of the drug in solution is reduced in the presence of HPMC.

$$\mu_i = \left(\frac{\partial G}{\partial N_i}\right)_{T,P,N_{j\neq i}} \qquad \text{Equation 2}$$

8.2. Observed Supersaturation of Compounds in the Presence of HPMC

In the supersaturation/kinetic solubility experiments in the presence of 5% HPMC E3 LV, all four of the compounds (NAP, IND, IBU, and ETO), were shown to maintain supersaturation for up to 3 hours. The alteration in the crystalline form of the precipitate and in most cases the amorphous character of the precipitate suggests that HPMC molecules may adsorb onto the surface of a solid precipitate preventing the initiation of crystallization to their most apparent stable form or, perturbing the growth of a seed crystals or preventing the formation of crystalline material altogether. The precipitated amorphous solid form has a higher free energy than the initial crystalline solid form, and thus when the solid form is amorphous the

solubility in solution increases. In the absence of HPMC, the precipitation of the compounds remains highly crystalline, and supersaturation is not maintained. The data suggests that supersaturation is generated and maintained with HPMC as a crystallization inhibitor, yielding a solid form of higher free energy, which results in increased solubility.

There appears to be some correlation between higher degree of supersatursaturation maintained and increased amorphous character in the precipitated material. In the case of both ETO and IND, the precipitation in the presence of HPMC yields a completely amorphous material, and precipitation is not observed after 3 hours with these compounds in the presence of HPMC, and a degree of supersaturation of 5 is maintained. In the case of NAP and IBU, the precipitation in the presence of HPMC showed to be a mixture of crystalline and amorphous material, and precipitation was observed at the initial time point and the degree of supersaturation did not exceed 3 for both compounds.

8.2.1. Varying Grade and Molecular Weight of HPMC Resulted in Some Differences in Etodolac Crystalline Equilibrium Solubility and Supersaturation

The data indicates that the presence of HPMC is more important than the molecular weight or grade. HPMC appears to enhance supersaturation in etodolac under acidic conditions by inhibiting crystallization and forming amorphous solid. The formation of amorphous solid could be a result of polymer molecules adsorbing onto the surface of a precipitate. The exact chemistry of HPMC or the viscosity of HPMC-containing solutions does not appear to affect the ability of etodolac to generate and maintain supersaturation per se. There might be some indication that the grade affects the amount of amorphous material that is formed, as precipitation in the presence of 5% HPMC E3 LV is completely amorphous, while precipitation in the presence of 5% HPMC E5 LV or 5% HPMC K3 LV show some crystallinity.

8.2.2. Increased Initial Degrees of Supersaturation Resulted in Decreased Time of Observed Precipitation of Etodolac

Under acidic conditions with pre-dissolved 5% HPMC K3 LV, an increased initial degree of supersaturation (within the range of 5-10 studied) appears to be a driving force for precipitation of etodoloc. At increasing degrees of supersaturation the solution free energy of etodolac increases proportionally, which increases the driving force for precipitation and as the degree of

supersaturatin increases the time to precipitate decreases. Also, once a seed crystal or amorphous solid is formed in a highly supersaturated solution the chance of redissolution is reduced and the chance of further solid growth is increased given the increased number of nucleation sites.

8.3. Mechanism of Supersaturation in Compounds in the Presence of HPMC is Increased Equilibrium Solubility and Crystallization Inhibition

In the cases of naproxen, indomethacin, and ibuprofen there is a significant relative increase in equilibrium solubility in the presence of 5% HPMC E3 LV without a change in crystalline form. The relative increase of equilibrium solubility in the presence of 5% HPMC E3 LV of etodolac was less, compared to the other compounds. In kinetic solubility experiments in the presence of 5% HPMC E3 LV, the harvested precipitates showed altered crystalline states or amorphous character relative to the initial crystalline form. This suggests that the mechanism of supersaturation in this series of compounds is a combination of increased equilibrium solubility and crystallization inhibition in the presence of HPMC.

The case of etodolac is different in that there was only a slight relative increase in equilibrium solubility in the presence of HPMC. However, the precipitation from kinetic solubility experiments show amorphous characteristics, suggesting that etodolac relies largely on crystallization inhibition to sustain supersaturation in the presence of HPMC.

8.4. Signifigance of Research

According to the findings, the presence of HPMC appears to provide favorable conditions for solubilizing and generating supersaturation in the neutral form of the evaluated crystalline carboxylic acids. The solubilizing effect of HPMC could be expanded and applied in the formulation of other poorly water-soluble compounds. HPMC could be added within a tablet formulation to provide supersaturation conditions upon *in vivo* oral administration and potentially improving oral bioavailability. The HPMC could also be further co-processed with the drug to generate amorphous solid dispersion material using a spray-drying manufacturing process. The work also confirms the conclusions of Ouellet et al[2], that the use of HPMC capsules can increase the oral bioavailability of a poorly water-soluble compound compared to gelatin capsules.

9. CONCLUSIONS

Supersaturation was shown to be maintained to varying extents in four structurally-related carboxylic acids drugs (naproxen, indomethacin, ibuprofen, and etodolac) under aqueous acidic conditions in the presence of HPMC. In three of the four compounds (naproxen, indomethacin, and ibuprofen), the mechanism by which supersaturation occurred was both by increased equilibrium solubility, and alteration in crystal form or crystallization inhibition in the presence of HPMC. Precipitates in the presence of HPMC often showed amorphous character, indicating a form change. In etodolac, there was only a small observed relative increase in equilibrium solubility, but a loss of crystallinity in the kinetic solubility studies, suggesting that etodolac sustains supersaturation largely by crystallization inhibition. Further studies with etodolac showed that the molecular weight or grade of HPMC has some effect on the degree of supersaturation but that the effect could not be correlated to chemistry or the viscosity of the solution. For etodolac over the range of 5-10 studied in the initial degree of supersaturation, an initial degree of supersaturation of 5 was optimal in maintaining supersaturation. Increased initial degrees of supersaturation resulted in decreased onset time of precipitation. The studies suggest that HPMC could potentially increase oral bioavailabilty by generating supersaturation of a poorly water-soluble compound upon *in vivo* oral administration.

Additional contributions could be made in further investigating the interactions between HPMC and the compounds. Each compound showed different maintained degrees of supersaturation, possibly due to different interaction strengths with HPMC. A correlation could be determined between the quantification of crystallinity in precipitate and the degree of supersaturation over a time-course experiment. Further evaluations could be made with ETO, to explore its interactions between the different grades of HPMC, as different grades showed different levels of crystallinity in the precipitate.

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